

Endothelial microparticles act as novel diagnostic and therapeutic biomarkers of circulatory hypoxia-related diseases: a literature review

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Abstract

Circulatory hypoxia-related diseases (CHRDs), including acute coronary syndromes, stroke and organ transplantation, attract increased attention due to high morbidity and mortality. Mounting evidence shows that hypoxia-induced oxidative stress, coagulation, inflammation and angiogenesis play extremely important roles in the physiological and pathological processes of CHRD-related vascular endothelial injury. Interestingly, hypoxia, even hypoxia-induced oxidative stress, coagulation and inflammation can all induce release of endothelial microparticles (EMPs). EMPs, shed from activated or apoptotic endothelial cells (ECs), reflect the degree of EC damage, and elevated EMP levels are found in several CHRDs. Furthermore, EMPs, which play an important role in cell-to-cell communication and function, have confirmed pro-coagulant, proinflammatory, angiogenic and other functions, affecting pathological processes. These findings suggest that EMPs and CHRDs have a very close relationship, and EMPs may help to identify CHRD phenotypes and stratify the severity of disease, to improve risk stratification for developing CHRDs, to better define prophylactic strategies and to ameliorate prognostic characterization of patients with CHRDs. This review summarizes the known and potential roles of EMPs in the diagnosis, staging, treatment and clinical prognosis of CHRDs.

Keywords: endothelial microparticles • hypoxia • circulatory hypoxia • ischaemic • biomarkers

Introduction

Hypoxia is the lack of oxygen supply to the tissues, resulting in abnormal cell metabolism and function, as well as morphological

alterations in the pathological process [1]. The aetiology of hypoxia-related diseases (HRDs) is complex, and many causes can lead to a vicious cycle with further exacerbation and worsening of the disease. Arterial oxygen pressure drop causes hypoxic hypoxia, tissue blood flow reduction results in circulatory hypoxia, haemoglobin reduction leads to hemic hypoxia, and altered bio-oxidation of tissues causes dysoxidative hypoxia. These are the four major clinical HRD types.

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Gradually increasing clinical data indicate that incidence and mortality of CHRDs, including myocardial infarction (MI), heart failure (HF), stroke and organ transplantation, are rising with living standard improvement [2]. Oxidative stress, inflammation and coagulation are always the causes of disease exacerbation and worsening and constitute important targets for CHRDs treatment.

Interestingly, hypoxia, oxidative stress, inflammation, coagulation can cause different degrees of vascular endothelial injury and stimulate the release of endothelial microparticles (EMPs). The above conditions can induce the release of EMPs both *in vivo* and *in vitro*. EMP levels are elevated in several CHRDs [3], including acute coronary syndromes (ACS) [4], MI and stroke [5]. Furthermore, EMPs, as active players in cell-to-cell communication, have pro-coagulant, proinflammatory and angiogenic properties. This implies that EMPs can help to identify CHRD phenotypes and stratify the severity of disease, to improve risk stratification for developing CHRDs, to better define prophylactic strategies and to ameliorate prognostic characterization of patients with CHRDs. Furthermore, as a pathogenic role for EMPs is clearly emerging, the precise description of the mechanisms underlying their formation is likely to prove valuable in identifying much needed novel therapeutic targets.

Membrane-derived vesicles

Cell-derived vesicles can be defined as spherical particles enclosed by a phospholipid bilayer [6]. The dimensions of these structures vary and represent one of the characteristics that distinguish the three types of vesicles previously mentioned: exosomes typically range in size between 30 and 100 nm and are released upon fusion of the limiting membrane of multivesicular bodies with the cell surface, microparticles range between 100 and 1000 nm and are formed by the outward blebbing of the plasma membrane with subsequent release after proteolytic cleavage of the cytoskeleton, while apoptotic bodies are between 1 and 3 μm and are formed by indiscriminate plasma membrane blebbing and possess nuclear fragments and histones [7–9]. In addition, the way these vesicles are obtained and what they carry or contain between these vesicles are different [7].

EMPs

EMPs are 100- to 1000-nm anucleated vesicles formed following cytoskeletal and membrane reorganization, and released from apoptosis or activation of ECs into the extracellular milieu [10, 11]. Ischaemia, hypoxia, hyperglycaemia, bacterial lipopolysaccharides, thrombin, C-reactive protein, active oxygen cluster and urea were shown to induce the release of EMPs from ECs [12]. Previous studies found elevated EMP levels in several disease conditions associated with tissue ischaemia or hypoxia [3] such as ACS [13, 14] and stroke [5]. Previous findings suggested that acute hypoxia leading to endothelial stress [15] and subsequently apoptosis constitutes a danger signal in case of an acute ischaemic or hypoxic event and can also trigger tissue repair mechanisms [16]. Furthermore, certain

circulating EMP phenotypes are closely associated with diagnosis, stratification, treatment and prognosis of CHRDs.

EMP phenotypes

EMPs can be defined according to membrane glycoproteins, as they possess antigens constitutively expressed on ECs such as CD31. The characteristic surface markers of EMPs that indicate ECs origin are numerous and different, with most of them not being specific markers for ECs. Strategies that use a combination of marker protein derived from different cell types have been proposed to resolve these difficulties. Epitopes derived from different cell types used are the positive EC markers (*e.g.* CD31 and CD144) in combination with the absence of the platelet markers CD41 or CD42b [17].

As shown in Table 1 [18], the levels of CD31⁺ EMPs, CD105⁺ EMPs and CD144⁺ EMPs appear to increase in apoptotic ECs. In contrast, when the endothelium becomes functionally activated, CD62E⁺, CD54⁺ and CD106⁺ EMP levels are increased. The relative proportion of CD62E⁺/CD31⁺ EMPs rather than absolute levels, distinguishes EMPs released by activated ECs from those derived from apoptotic ECs. More than 10% CD62E⁺/CD31⁺ EMPs indicates that most originate from activated ECs, whereas 1% or less reflects an apoptotic origin [19].

Studies have confirmed that different stimuli can induce the release of different EMPs both *in vitro* and *in vivo*. Although the clinical significance of these differences in EMP release remains not entirely clear, the pattern of EMP release in response to a specific stimulus differs depending on the type of ECs [20]. As shown in Table 2, different EMP subtypes may play a role in CHRDs.

CD31⁺ EMPs

PECAM-1 EMPs, defined as CD31⁺ EMPs, are concentrated at endothelial junctions. However, the PECAM-1 marker is also expressed on the surface of platelets, neutrophils and lymphocyte subsets; therefore, platelet-specific markers, such as CD41 or CD42b, are used to distinguish CD31⁺ EMPs from platelet-derived MPs. CD31⁺ EMPs are defined as CD31⁺/CD41⁻ or CD42b⁻ EMPs [21, 22]. CD31⁺ EMPs may play diverse roles in vascular biology, regulating the platelet function, angiogenesis, T cell and B cell activation, EC permeability and transmigration across the endothelium [23–28]. Therefore, the released EMPs likely reflect the apoptosis of injured ECs.

CD51⁺ EMPs

Integrin- α V⁺ EMPs are defined as CD51⁺ EMPs. CD51 forms a heterodimer with an integrin- β 3 chain, such as glycoprotein IIIa (GPIIIa) or CD61, and binds to vitronectin, von Willebrand factor (vWF) and fibronectin [29]. CD51 is found on ECs, B lymphocytes, monocytes, macrophages and platelets [30]. CD51⁺ EMPs may play important roles in leucocyte homing and rolling and angiogenesis [28, 31].

CD54⁺ EMPs

ICAM-1 EMPs are CD54⁺ EMPs. ICAM-1 is the cognate ligand of lymphocyte function-associated antigen-1 (LFA-1), and a transmembrane protein found on the surface of inflamed ECs, epithelial cells,

Table 1 Endothelial markers used for detecting EMPs

CD marker	Antigen	Expression	Localization on ECs	Expression on ECs	[Refs]
CD31	PECAM-1	Apoptosis	Intercellular junction (outside of adherence junctions)	Constitutively expressed	[19, 88, 89]
CD51	Integrin- α v	Apoptosis	Surface	Constitutively expressed	[90]
CD54	ICAM-1	Activation	Surface		[89]
CD62E	E-selectin	Activation	Surface	Low on rested ECs; rapidly up-regulated in response to inflammation	[19, 89]
CD105	Endoglin	Apoptosis	Surface	Low on resting ECs; up-regulated once angiogenesis begins	[19, 89]
CD106	VCAM-1	Activation			[19, 89]
CD144	VE-cadherin	Apoptosis	Adherence junction	Constitutively expressed	[19]
CD146	MCAM	Apoptosis	Intercellular junction (outside of adherence junctions) and surface	Constitutively expressed	[54]

ECs, endothelial cells; EMPs, endothelial microparticles; PECAM, platelet endothelial cell adhesion molecule; MCAM, melanoma cell adhesion molecule; VE, vascular endothelial.

monocytes and macrophages, as well as other immune cells including T cells, B cells and antigen-presenting cells (APCs) [32–35]. The LFA-1/ICAM-1 interaction is critical to the firm adhesion of T cells to the vascular endothelium of inflamed tissues and influences the diapedesis and migration of these adhered lymphocytes out of the vasculature directly into adjacent tissues on the ocular surface [36].

CD62E⁺ EMPs

E-selectin is expressed only on ECs, and E-selectin EMPs are defined as CD62E⁺ EMPs. E-selectin is rapidly induced on activated ECs a few hours after inflammatory stimulation, whereas VE-cadherin, PECAM-1 and MCAM are constitutively expressed on ECs. CD62E⁺ EMPs may play an important role in recruiting leucocytes to the site of injury during inflammation [23–28]. In addition, tumour necrosis factor- α (TNF- α) was shown to induce the release of CD62E⁺ EMPs *in vitro* [20, 37]. Thus, CD62E⁺ EMP levels may reflect the degree of ongoing endothelial inflammation.

CD105⁺ EMPs

Endoglin EMPs are CD105⁺ EMPs. Endoglin (CD105) is a marker of endothelial and mesenchymal stem cells, and component of TGF- β , BMP-9 and BMP-10-binding receptor complexes [38]. CD105 is increasingly expressed on ECs during tumour angiogenesis and inflammation, but have low expression levels on normal ECs [39]. Its expression is significantly increased on blood vessel endothelium in ischaemic tissues [38].

CD144⁺ EMPs

VE-cadherin EMPs are CD144⁺ EMPs. VE-cadherin is expressed only on ECs and functions as a gatekeeper of endothelial junctions [28,

40]. Therefore, CD144 is the most specific marker for EMPs [41]. In contrast to PECAM-1 and MCAM, VE-cadherin is specifically located at adherence junctions [21]. In addition, changes in VE-cadherin localization are associated with neutrophil migration and increased vascular permeability [42]. The release of CD144⁺ EMPs may reflect the structural destruction of the endothelium rather than an inflamed lung.

CD146⁺ EMPs

MCAM EMPs are CD146⁺ EMPs. MCAM is an adhesion molecule found on ECs and located outside of adherence junctions. MCAM is not only expressed on ECs, but also found on other cell types [43], especially subsets of T and B lymphocytes as well as natural killer (NK) cells under pathological conditions [44], and pericytes [45]. Therefore, CD146⁺ EMPs, another indicator of ECs injury, may be closely related to signalling transduction, endothelial permeability, cell migration, angiogenesis and the immune response [46].

EMPs as messengers

Although EMPs only represent 5–15% of all MPs, they play a vital role in cell-to-cell communication, which impacts biological activity. Not only can they be used as a biomarker to distinguish different stimulating factors, disease status and prognosis, they can actively alter the pathological process and disease development.

EMPs and prothrombotic response

A study identified an original pathway of EMP release induced by thrombin, involving activation of the Rho-kinase ROCK-II by

Table 2 The different EMPs related to CHRDS

EMPs	Diseases	Aspects related to diseases	[Refs]
CD31 ⁺	CAD	Coronary endothelial function, cardiovascular events, risk stratification, LV dysfunction and endothelium-dependent vasodilation	[13,64–66]
	ACS	Acute endothelial injury, risk stratification	[54,69]
	MI	Recurrence, the size of MaR in STEMI patients, thrombus occlusion	[13,14,71]
	Cerebrovascular atherosclerosis	The EMP level significantly discriminates extracranial and intracranial arterial stenosis	[78]
CD51 ⁺	CAD	Assessing EC injury	[13]
CD54 ⁺	MI	Treatment with omega-3	[72]
	Acute stroke	Severity, lesion volume and outcome of acute ischaemic stroke	[5]
CD62E ⁺	MI	Thrombus occlusion	[71]
	Aortic stenosis	Systemic inflammatory activity, activated monocytes	[91]
	Acute stroke	Stroke severity, infarct volume, the risk for cardiovascular morbidities, recurrent events	[78,80]
	Cerebrovascular atherosclerosis	The EMP level significantly discriminates extracranial and intracranial arterial stenosis	[78]
	Heart transplant	As an independent marker of acute allograft rejection	[84]
CD105 ⁺	MI	Angiographic lesions, coronary endothelial damage, restoration of the epicardial blood flow	[70]
	Acute stroke	Severity, lesion volume and outcome of acute ischaemic stroke	[5]
	SAH	Cerebral vasospasm	[79]
CD144 ⁺	CAD	Intermediate lesion	[67]
	CHF	An independent predictor of future cardiovascular events	[74]
	Coronary artery stenosis	Cardiac ischaemia	[92]
	Acute stroke	Severity, lesion volume and outcome	[5]
	Heart transplant	An independent predictor of cardiac allograft vasculopathy	[85]
	Liver transplant	Clinical outcome	[83]
CD146 ⁺	ACS	Acute endothelial injury	[54]
	MI	Ischaemic time	[71]

CAD, coronary artery disease; LV, left ventricular; ACS, acute coronary syndrome; MI, myocardial infarction; STEMI, ST-segment elevation myocardial infarction; MaR, myocardium at risk; ECs, endothelial cells; SAH, spontaneous subarachnoid haemorrhage; CHF, congestive heart failure.

caspase 2 in the absence of cell death [47]. This mechanism of EMP generation depends on the activation of nuclear factor (NF)- κ B, whose signalling pathway controls thrombin-mediated up-regulation of the inflammatory mediators ICAM-1 and IL-8 [48].

Thrombin induces the release of EMPs, which in turn have the ability to mediate thrombin generation in many diseases. PS can bind coagulation factors and promote their activation, which may explain

why PS⁺ EMPs have pro-coagulant properties. In addition, EMPs expressing tissue factor (TF), which is the initiator of the extrinsic coagulation pathway, induce TF-dependent thrombin formation *in vitro* and *in vivo* [49, 50]. Furthermore, TF⁺ EMPs bind to monocytes and platelets, amplifying proinflammatory and pro-coagulant responses [51]. Pro-coagulant EMPs have also been found in atherosclerotic plaques [52, 53] and in patients with ACS [54].

Therefore, EMPs can potentially behave as biological vectors in the dissemination of pro-coagulant potential.

EMPs and proinflammatory response

Experiments in cultured ECs have indicated that EMP release is related with that of proinflammatory factors, indicating that release of EMPs and classical inflammatory pathways may have a close relationship in CHRDs [50]. Additionally, the interaction between EMPs and ECs triggers proinflammatory responses by increasing ICAM messenger RNA (mRNA) expression as well as soluble ICAM shedding from target cells [55]. EMP can amplify the bidirectional relationship between inflammation and thrombosis by conveying cell information. Hence, EMPs represent both a cause and a consequence of the inflammatory response.

EMPs as angiogenic signals

Besides their pro-coagulant and proinflammatory activities, EMPs are also involved in surface supporting plasmin generation by expressing urokinase-type plasminogen activator (uPA) and its receptor (uPAR), which could counteract EMP generated thrombin and play a pivotal role in maintaining vascular patency and facilitating cell migration and angiogenesis [56, 57]. By conveying plasmin, EMPs activate matrix metalloproteinases, which are involved in extracellular matrix degradation and the release of growth factors that play pivotal roles in tissue remodelling and angiogenesis. Consistently, EMPs derived from ischaemic muscle are known to promote endothelial proliferation and vasculogenesis, and TF-rich EMPs released by microvascular ECs can overcome the consequences of arterial occlusion and tissue ischaemia by promoting post-ischaemic neovascularization and tissue reperfusion [58, 59].

However, the involvement of EMPs in the angiogenic response and vascular repair remains controversial. In contrast, EMPs reduce endothelial proliferation as well as the formation of new vessels *in vitro* [60]. Similarly, high levels of EMPs isolated from human umbilical vein ECs were shown to abolish angiogenesis, while low EMP amounts promote the formation of capillary-like structures [61]. In addition, angiogenic response promotion may also have deleterious effects in cancer spreading, proliferative diabetic retinopathy [62] or atherosclerotic plaque destabilization by promoting intraplaque neovascularization [63].

EMPs in CHRDs

In the normal physiological state, oxygen in the air flows from the respiratory tract into the alveoli, diffuses to the blood, binds to haemoglobin and is transported by the blood circulation to the body for use by tissue cells. Obstruction of any of these processes can lead to hypoxia. Circulatory hypoxia, including heart failure, shock, embolism and arterial stenosis, represents a decrease in tissue blood flow resulting in tissue oxygen deficiency. EMPs have been investigated in several human circulatory hypoxia diseases as possible pathogenic factors, prognostic markers and therapeutic targets. The data reported in the following sections are summarized in Table 3.

EMPs and coronary artery disease (CAD)

The vascular endothelium and EMPs play pivotal roles in the pathogenesis and clinical manifestations of CAD. Many groups have demonstrated that EMP levels increase significantly in patients with CAD compared with controls [13, 64]. Sinning *et al.* found that CD31⁺/AV⁺ EMPs are significantly higher in patients with major adverse cardiovascular and cerebral events (MACCEs) compared with those without such events [65]. Furthermore, Bulut *et al.* demonstrated that CD31⁺/AV⁺ EMP levels are significantly higher in stable CAD (SCAD) patients with left ventricular (LV) dysfunction compared with those with normal or preserved LV function [66]. These findings imply that circulating CD31⁺/AV⁺ EMP levels constitute an independent predictor of cardiovascular events in patients with SCAD and may be useful for risk stratification; meanwhile, circulating CD31⁺/AV⁺ EMP levels are inversely correlated with endothelium-dependent vasodilation in patients with SCAD. In addition, Song *et al.* found that CD144⁺ EMP levels are significantly higher in patients with intermediate lesions compared with controls, which suggests that CD144⁺ EMPs level can reflect the severity of coronary stenosis in patients with CAD [67].

In addition, the levels of EMP-platelet aggregates (EMP-P) were found to be significantly higher in patients with SCAD than controls and early acute myocardial infarction (AMI) patients. EMP-P may play a role in thrombus generation through their pro-coagulant activity [68].

EMPs and ACS

Rupture or plaque fissuring promotes adhesion of platelets and leucocytes and exposes the tissue to promote thrombus formation and key events in the pathogenesis of acute coronary syndromes (ACS). Mallat *et al.* demonstrated that high levels of pro-coagulant EMPs (CD31⁺ EMPs and CD146⁺ EMPs) are present in the circulating blood of patients with ACS and may contribute to the generation and perpetuation of intracoronary thrombi. In addition, the presence of CD31⁺ EMPs and CD146⁺ EMPs suggest a potentially important role for acute endothelial injury in ACS [54]. Bernal-Mizrachi *et al.* found that CD31⁺/CD42b⁻ EMP levels are significantly higher in ACS patients with high-risk lesions than those with low-risk lesions, in lesions with thrombi compared with those without, in ACS patients with mild stenosis compared with those with severe or without stenosis, and in first ACS with high-risk lesions than those with recurrent ACS [69]. These findings imply that CD31⁺/CD42b⁻ EMPs may constitute a useful marker in detecting endothelial injury and risk of ACS as defined by angiography.

EMPs and MI

Endothelial activation and dysfunction are prominent features of acute myocardial infarction (MI); mounting evidence shows EMPs play an important role in diagnosis and differential diagnosis of MI. Jung *et al.* found that ST-segment elevation myocardial infarction (STEMI) patients with left anterior descending artery (LAD) occlusion have higher levels of CD31⁺/CD42⁻ EMP than those with other infarct-related arteries, while CD144⁺ EMP does not differ between the two

Table 3 The EMPs in circulatory hypoxia diseases

Diseases	Changes of EMPs	Major finding	[Ref.]
CAD	CD31 ⁺ /AV ⁺ EMPs ↑ (patients <i>versus</i> controls)	Increased CD31 ⁺ /AV ⁺ EMP counts positively correlated with impairment of coronary endothelial function	[64]
	CD31 ⁺ /AV ⁺ EMPs ↑ (cardiovascular events in stable CAD patients <i>versus</i> controls)	The level of circulating CD31 ⁺ /AV ⁺ EMPs is an independent predictor of cardiovascular events in stable CAD patients and may be useful for risk stratification	[65]
	CD144 ⁺ EMPs ↑ (patients with intermediate lesions <i>versus</i> controls)	The levels of CD144 ⁺ EMPs are significantly higher in the intermediate lesion group	[67]
	CD31 ⁺ /AV ⁺ EMPs ↑ (stable CAD patients with LV dysfunction <i>versus</i> those with normal or preserved LV function)	CD31 ⁺ /AV ⁺ EMPs correlated inversely with endothelium-dependent vasodilation in stable CAD patients	[66]
	CD31 ⁺ EMPs ↑ & CD51 ⁺ EMPs ↑ (patients <i>versus</i> controls)	EMP assay appears promising for assessing EC injury in CAD	[13]
	EMP-P ↑ (stable CAD patients <i>versus</i> controls; stable CAD patients/controls <i>versus</i> within 1 hr of AMI patients); EMP-P ↔ (48 hr after the onset of AMI <i>versus</i> stable CAD)	Microparticles shed from activated endothelial cells can interact with platelets and form aggregates <i>in vitro</i> and <i>in vivo</i>	[68]
ACS	CD31 ⁺ EMPs ↑ & CD146 ⁺ EMPs ↑ (patients <i>versus</i> stable coronary/non-coronary patients)	CD31 ⁺ EMPs and CD146 ⁺ EMPs suggest a potentially important role for acute endothelial injury in ACS	[54]
	CD31 ⁺ EMPs ↑ & CD51 ⁺ EMPs ↑ (patients <i>versus</i> controls); CD31 ⁺ EMPs ↑ & CD51 ⁺ EMPs ↔ (ACS patients <i>versus</i> SA controls)		[13]
	CD31 ⁺ /CD42b ⁻ EMPs ↑ (ACS patients with high-risk lesions <i>versus</i> low-risk lesions; lesions with thrombi <i>versus</i> those without; mild stenosis <i>versus</i> severe/without stenosis; first ACS with high-risk lesions <i>versus</i> those with recurrent ACS)	CD31 ⁺ /CD42b ⁻ EMPs may be a useful marker in detecting endothelial injury and risk of ACS as defined by angiography	[69]
MI	CD31 ⁺ EMPs ↑ (first MI patients <i>versus</i> UA/recurring MI)		[13]
	CD31 ⁺ EMPs ↑ (patients <i>versus</i> controls)		[93]
	CD105 ⁺ EMPs ↑ (the occluded coronary artery of STEMI patients <i>versus</i> peripheral blood); CD105 ⁺ EMPs ↓ (restoration of the epicardial blood flow <i>versus</i> controls)	CD105 ⁺ EMPs are elevated in angiographic lesions and correlated with coronary endothelial damage	[70]
	CD31 ⁺ /CD42 ⁻ EMPs ↑ & CD144 ⁺ EMPs ↔ (patients with LAD infarctions <i>versus</i> those with other infarct-related arteries)	Circulating CD31 ⁺ /CD42 ⁻ EMPs correlate to the size of MaR in patients with STEMI	[14]
	AV ⁺ EMPs ↑ (STEMI patients <i>versus</i> controls; patients in the acute phase and in peripheral blood) CD62E ⁺ /AV ⁺ EMPs ↑ & CD31 ⁺ /AV ⁺ ↓ (peripheral circulation of STEMI patients <i>versus</i> controls); CD62E ⁺ /AV ⁺ EMPs ↑ & CD146 ⁺ /AV ⁺ EMPs ↑ (intracoronary blood in STEMI patients <i>versus</i> those in peripheral blood); intracoronary CD146 ⁺ EMPs ↑ (IT ≤3 hr <i>versus</i> IT >3 hr)	EMPs in peripheral blood may be sensitive markers of the thrombo-occlusive vascular process developing in the coronary arteries of STEMI patients	[71]
	CD54 ⁺ EMPs ↓ (MI in rats with omega-3 treatment <i>versus</i> controls)		[72]

Table 3. Continued

Diseases	Changes of EMPs	Major finding	[Ref.]
CHF	CD62E ⁺ EMPs ↑ & CD31 ⁺ EMPs ↑ (patients <i>versus</i> controls/post-transplant patients); CD62E ⁺ EMPs ↔ (patients <i>versus</i> post-transplant patients)	Cardiac transplantation is associated with a different pattern of endothelial cell injury than that seen in heart failure. The phenotypic assessment of EMPs in post-transplant patients is consistent with increased apoptotic activity	[73]
	CD144 ⁺ EMPs ↑ (patients <i>versus</i> controls)	High-CD144 ⁺ EMP levels are an independent predictor of future cardiovascular events	[74]
CPR	EMP-P ↑ & EMP-M ↑ (immediately after CPR <i>versus</i> controls); EMP-M ↑ (24 hr after CPR <i>versus</i> controls)		[76]
	CD62E ⁺ EMPs ↑ (24 hr after CPR <i>versus</i> controls); CD62E ⁺ EMPs ↔ (immediately after CPR <i>versus</i> controls)	An ongoing process of endothelial injury, paralleled by a subsequent endothelial regeneration 24 hrs after resuscitation	[75]
Severe aortic stenosis	CD62E ⁺ EMPs ↑ (patients <i>versus</i> controls)	EMPs reflecting the activation of ECs but also conferring systemic inflammatory activity were increased in severe aortic stenosis patients and correlated with the number of activated monocytes	[91]
Coronary artery stenosis	CD144 ⁺ EMPs ↓ (immediately after dobutamine stress echocardiography in patients <i>versus</i> controls)	Cardiac ischaemia leads to reduced circulating EMP levels under cardiac stress	[92]
Acute stroke	CD105 ⁺ /CD144 ⁺ EMPs ↑ & CD105 ⁺ /CD54 ⁺ EMPs ↑ & CD105 ⁺ /PS ⁺ EMPs ↑ (patients <i>versus</i> controls)	Certain circulating EMP phenotypes may be associated with severity, lesion volume and outcome of acute ischaemic stroke	[5]
	CD62E ⁺ EMPs ↑ & CD31 ⁺ /CD42b ⁻ EMPs ↑ & CD31 ⁺ /AV ⁺ EMPs ↑ (patients <i>versus</i> controls with risk factors)	The levels of CD62E ⁺ EMPs were strongly associated with stroke severity and infarct volume	[78]
	CD62E ⁺ EMPs ↑ (patients <i>versus</i> controls)	Elevated CD62E ⁺ EMP levels increase the risk for cardiovascular morbidities	[80]
	CD62E ⁺ /CD42a ⁻ /AV ⁺ EMPs ↑ & CD31 ⁺ /CD42a ⁻ /AV ⁺ EMPs ↑ (recurrent childhood arterial ischaemic stroke <i>versus</i> those with no recurrent/controls/cerebral arteriovenous malformation)	Endothelial injury and cellular activation are different in patients with single and recurrent events	[94]
	CD62E ⁺ EMPs ↔ & CD31 ⁺ EMPs ↔ (stroke mimic patients <i>versus</i> acute ischaemic stroke)	EMPs may not be a good marker for acute ischaemic stroke, given the inability to discriminate between stroke mimics and acute ischaemic stroke	[82]
	CD146 ⁺ /AV ⁺ EMPs ↑ & CD62E ⁺ /AV ⁺ EMPs ↑ & CD146 ⁺ /CD62E ⁺ /AV ⁺ EMPs ↑ (acute ischaemic stroke patients <i>versus</i> high cardiovascular risk controls)		[81]

Diseases	Changes of EMPs	Major finding	[Ref.]
Cerebrovascular atherosclerosis	CD62E ⁺ EMPs ↑ & CD31 ⁺ EMPs ↑ (patients <i>versus</i> controls); CD62E ⁺ EMPs ↑ & CD31 ⁺ /CD42b ⁻ EMPs ↓ & CD31 ⁺ /AV ⁺ EMPs ↓ (patients with extracranial arterial stenosis <i>versus</i> patients with intracranial arterial stenosis)	The ratio of CD62E ⁺ to CD31 ⁺ /CD42b ⁻ or CD31 ⁺ /AV ⁻ EMP level significantly discriminated extracranial and intracranial arterial stenosis	[78]
Cerebral Ischaemia	CD31 ⁺ /CD42b ⁻ EMPs ↔ (patients <i>versus</i> controls)		[77]
SAH	CD105 ⁺ EMPs ↑ & CD62E ⁺ EMPs ↑ (patients with cerebral vasospasm <i>versus</i> controls)	The elevated CD105 ⁺ EMPs could be a novel biomarker for cerebral vasospasm in SAH patients	[79]
DIC	CD105 ⁺ EMPs ↑ & CD31 ⁺ EMPs ↑ (Septic shock-induced DIC <i>versus</i> controls)	The EMPs are relevant biomarkers of septic shock-induced DIC and could be used to evaluate early vascular injury	[95]
Liver Transplant	CD144 ⁺ EMPs ↑ (patients <i>versus</i> patients with a partial hepatectomy)	The levels of circulating EMPs in liver transplant patients dynamically change after surgery and correlate with the clinical outcome	[83]
Hepatic I/R	AV ⁻ EMPs ↑ (4 hr after reperfusion <i>versus</i> controls); AV ⁻ EMPs ↑ (between 48 and 72 hrs after reperfusion <i>versus</i> controls);	EMPs increase after the injury response during the reparative phase and may be important in angiogenesis that occurs in the regenerating liver	[96]
Heart transplant	CD62E ⁺ EMPs ↑ & CD62E ⁺ EMPs/CD31 ⁺ EMPs ↑ & CD31 ⁺ EMPs ↔ (patients with rejection <i>versus</i> controls) CD31 ⁺ EMPs ↑ (patients <i>versus</i> controls)	CD62E ⁺ EMPs appeared as an independent marker of acute allograft rejection	[84]
Renal transplant	CD144 ⁺ /CD42a ⁻ EMPs ↑ (patients <i>versus</i> controls; patients with CAV <i>versus</i> those without CAV) CD31 ⁺ EMPs ↑ (patients with cyclosporine treatment <i>versus</i> controls) CD31 ⁺ /CD42b ⁻ EMPs ↓ (patients with selective end-stage kidney disease <i>versus</i> controls)	CD144 ⁺ /CD42a ⁻ EMPs have the high discriminative ability between CAV-positive and CAV-negative in heart transplant patients. Cyclosporine causes endothelial cells to release complement-activating CD31 ⁺ EMPs <i>in vivo</i>	[73] [85] [86] [87]

AV, annexin V; CAD, coronary artery disease; LV, left ventricular; ACS, acute coronary syndrome; MI, myocardial infarction; AMI, acute myocardial infarction; STEMI, ST-segment elevation myocardial infarction; SA, stable angina; UA, unstable angina; MaR, myocardium at risk; ECs, endothelial cells; CHF, congestive heart failure; EMP-P, endothelial microparticle-platelet aggregates; EMP-M, endothelial microparticle-monocyte aggregates; CPR, cardiopulmonary resuscitation; LAD, left anterior descending artery; SAH, spontaneous subarachnoid haemorrhage; CAV, cardiac allograft vasculopathy; DIC, disseminated intravascular coagulation; ↑, increased; ↔, unchanged; ↓, decreased.

groups; meanwhile, CD31⁺/CD42⁻ EMP amounts are correlated to myocardium at risk (MaR) and the area under the curve of troponin T levels, but not to infarct size (IS) and CKMB [14]. In patients with first MI, CD31⁺ EMP levels were shown to be higher than in those with UA, and significantly elevated compared with the values obtained for patients with recurring MI [13]. Morel *et al.* found that CD105⁺ EMP levels are significantly higher in occluded coronary artery specimens than in peripheral blood samples, with restoration of the epicardial blood flow leading to significantly reduced CD105⁺ EMP levels; these findings suggest that CD105⁺ EMPs are elevated in angiographic lesions and correlated with coronary endothelial damage [70]. Interestingly, Suades *et al.* found that AV⁺ EMPs are significantly increased in the systemic circulation of STEMI patients compared with controls, with lower amounts of AV⁺ EMPs in peripheral blood of patients recovering from MI at day 3 than in individuals with acute phase disease. The levels of CD62E⁺/AV⁺ EMPs were shown to be significantly higher, with CD31⁺/AV⁺ EMP amounts significantly lower in peripheral circulation of STEMI patients than controls. In addition, CD62E⁺/AV⁺ EMP levels and CD146⁺/AV⁺ EMP amounts are significantly higher in intracoronary blood than in peripheral blood; meanwhile, CD146⁺/AV⁺ EMP amounts were shown to be significantly higher in patients with ischaemic time (IT) ≤ 3 hr than in those with IT > 3 hr [71]. These findings imply that EMPs in peripheral blood may be sensitive markers of the thrombo-occlusive vascular process developing in the coronary arteries of STEMI patients. Interestingly, omega-3 bolus before reperfusion significantly decreases I/R injury and CD54⁺ EMP release compared with untreated I/R rats [72]. Therefore, CD54⁺ EMPs may constitute a good marker for the treatment of prognosis in MI.

EMPs and CHF

EMPs are reportedly elevated in patients with congestive heart failure (CHF). Garcia *et al.* found CHF patients have significantly higher levels of CD62E⁺ EMP than controls and post-transplant patients. CD31⁺ EMP levels in CHF patients are significantly higher than in controls. In addition, CHF patients and controls have significantly higher ratios of CD62E⁺ EMPs to CD31⁺ EMPs than post-transplant patients; no significant differences were found between CHF patients and controls [73]. This implies that EMPs may help in differential diagnosis between CHF and heart transplant. Furthermore, Nozaki *et al.* demonstrated that plasma levels of CD144⁺ EMPs can independently predict future cardiovascular events in patients with HF, constituting a potentially useful biomarker of endothelial dysfunction in HF risk stratification [74].

EMPs and CPR

Ischaemia and reperfusion after cardiopulmonary resuscitation (CPR) induce endothelial activation and systemic inflammatory response, resulting in post-resuscitation disease. Fink *et al.* found that CD62E⁺ EMP amounts are slightly and non-significantly increased immediately after CPR compared with values of patients with CAD or healthy controls until 24 hrs after successful CPR [75]. In resuscitated patients, significantly enhanced levels of EMP–monocyte and EMP–

platelet conjugates were obtained compared with patients in the cardiologic control group and healthy controls immediately after cardiopulmonary resuscitation (CPR); meanwhile, only the levels of EMP–monocyte conjugates show persisting increase within 24 hrs of CPR [76]. The above findings indicated that early endothelial damage and ongoing endothelial dysfunction lead to the release of apoptotic EC-derived EMPs, and more activating cell-derived EMPs are increased, which may be related to early endothelial cell regeneration, 24 hrs after CPR.

EMPs and acute stroke (AS)

Cerebral ischaemia is a major complication after acute brain injury, in which endothelial dysfunction is a key player [77]. Detailed profiling of EMPs may help better understand the pathogenesis of stroke and determine the related risks. Cerebrovascular atherosclerosis, ischaemia and vasospasm all can increase EMPs to different degrees [77–79]. Simak *et al.* found that certain circulating EMPs may be associated with severity, lesion volume and outcome in acute ischaemic stroke (AIS). Significantly higher CD105⁺/PS⁺/CD41a⁻ EMP counts were observed in AIS patients compared with controls, with three EMP phenotypes (CD105⁺/CD41a⁻/CD45⁻ EMPs, CD105⁺/PS⁺/CD41a⁻ EMPs and CD105⁺/CD54⁺/CD45⁻ EMPs) correlating significantly with brain lesion volume, and CD105⁺/CD54⁺/CD45⁻ EMPs showing the strongest correlation. Admission counts of CD105⁺/CD144⁺ EMPs and CD105⁺/CD41a⁻/CD45⁻ EMPs were correlated significantly with discharge clinical outcome. However, these changes are different between AIS and moderate to severe stroke; all four EMP phenotypes (CD105⁺/CD41a⁻/CD45⁻ EMPs, CD105⁺/CD144⁺ EMPs, CD105⁺/PS⁺/CD41a⁻ EMPs and CD105⁺/CD54⁺/CD45⁻ EMPs) studied were shown to be elevated in the subgroup of moderate to severe stroke patients compared with controls [5]. Jung *et al.* demonstrated that CD62E⁺ EMP levels are strongly associated with stroke severity and infarct volume. Furthermore, CD62E⁺ EMP levels were found to be negatively correlated with time since symptom onset and positively associated with National Institutes of Health Stroke Scale (NIHSS) scores. In addition, EMP profiling is important for the diagnosis of intracranial arterial stenosis (ICAS) and extracranial arterial stenosis (ECAS). The ICAS subgroup was shown to have greater CD31⁺/CD42b⁻ and CD31⁺/AV⁺ EMP levels and a lower CD62E⁺ to CD31⁺ to AV⁺ EMP ratio compared with the other subgroups. In contrast, the ECAS subgroup show greater mean CD62E⁺ EMP levels and CD62E⁺ to CD31⁺/AV⁺ EMP ratio compared with the other subgroups [78]. Furthermore, Lee *et al.* found that high level of CD62E⁺ EMPs is associated with cardiovascular events in patients with stroke history, suggesting that systemic endothelial activation increases the risk for cardiovascular morbidities [80]. Consistently, Chiva-Blanch *et al.* found that CD146⁺/AV⁺ EMP and CD62E⁺ EMP levels are significantly higher in AIS patients than high cardiovascular risk controls [81]. These findings suggest that different EMPs may have distinct biological significances in the pathogenesis of cerebral atherosclerosis, with different diagnostic values.

However, EMPs may not be a good marker for acute ischaemic stroke, given the inability to discriminate between stroke mimics and

acute ischaemic stroke. B. Williams *et al.* found CD62E⁺ EMP and CD31⁺ EMP levels as well as the CD62E⁺ EMPs to CD31⁺ EMPs ratio are similar in patients with AIS and stroke mimic patients [82].

EMPs and organ transplantation

Mounting evidence shows that the levels of circulating EMPs may constitute a biomarker for evaluating the functional status of transplanted organs in clinical practice. Brodsky *et al.* found that the levels of CD144⁺ EMPs are increased more substantially in patients who underwent liver transplantation compared with those administered partial hepatectomy, while circulating EMP levels in liver transplantation patients dynamically change after surgery and correlate with the clinical outcome [83].

In heart transplantation patients, the levels of CD31⁺ EMPs are significantly higher than in controls [73], and CD62E⁺ EMPs appeared to be an independent predictive factor for acute allograft rejection. Morel *et al.* found that CD62E⁺ EMP levels and the ratio of CD62E⁺ EMPs to CD31⁺ EMPs are significantly higher in heart transplantation patients with rejection than those with no rejection, whereas apparently similar CD31⁺ EMP levels were found [84]. Cardiac allograft vasculopathy (CAV) is the most important determinant of cardiac allograft survival, and a major cause of death after heart transplantation. Singh *et al.* found the levels of CD144⁺/CD42a⁻ EMPs are significantly higher in heart transplantation patients compared with controls, and higher in heart transplantation patients with CAV than those without. These findings indicated that CD144⁺/CD42a⁻ EMPs have a high discriminative ability between CAV-positive and CAV-negative heart transplant patients [85].

In kidney transplantation patients, circulating EMP levels 1 year after transplantation were shown to be significantly lower than pre-transplantation values. Interestingly, the levels of EMPs in post-transplantation patients administered cyclosporine and azathioprine immunosuppressive treatment were shown to be significantly lower

than those receiving tacrolimus and mycophenolate immunosuppressive treatment. These findings suggest that renal transplant-related injuries induced release of EMPs is an important mechanism by which systemic insults trigger intravascular complement activation and complement-dependent renal diseases [86]. In addition, circulating EMP levels in renal transplantation patients with selective end-stage kidney disease are significantly reduced compared with controls. Circulating EMP amounts change with positive and negative peritubular capillary C4d staining on kidney allograft biopsy, again indicating that circulating EMPs constitute a good biomarker for reflecting EC injury in various diseases [87].

Conclusions

Although knowledge regarding EMPs in the development of clinical disorders is not completely clear, with potential causal or effect relationships between EMP release and particular disease processed still being unravelled, there is little doubt that EMPs are good biomarkers for identifying CHRd phenotypes and assessing disease severity, improving risk stratification for the development of CHRds to better define prophylactic strategies and allowing a better prognostic characterization of patients with CHRds.

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Conflict of interest

The authors declare that they have no conflict of interest.

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